

**IN THE CLAIMS:**

1. **(Currently Amended)** An isolated nucleic acid fragment selected from the group consisting of:

(a) an isolated nucleic acid fragment encoding a polypeptide with nitrilase activity [selected from the group consisting of ] as set forth as SEQ ID NO:5 [and SEQ ID NO:14];

(b) an isolated nucleic acid molecule that hybridizes with the isolated nucleic acid fragment of (a) under stringent hybridization conditions of 6X SSC (1M NaCl), 40 to 45 % formamide, 1 % SDS at 37 °C, and a wash in 0.5X to 1X SSC at 55 to 60 °C; and

(c) an isolated nucleic acid fragment that is completely complementary to (a) or

(b).

2. **(Currently Amended)** An isolated nucleic acid fragment comprising a first nucleotide sequence encoding a polypeptide with nitrilase activity ~~that has greater than 71 %~~ having at least 80 % identity based on as determined by the Needleman and Wunsch algorithm under default parameters when compared to a polypeptide encoded by the sequence identified in SEQ ID NO:5, or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

3. **(Currently Amended)** An isolated nucleic acid fragment encoding a nitrilase enzyme, selected from the group consisting of:

(a) an isolated nucleic acid fragment selected from the group consisting of SEQ ID NO:4, [SEQ ID NO:13,] and SEQ ID NO:15 [and SEQ ID NO:16];

(b) an isolated nucleic acid molecule that hybridizes with the isolated nucleic acid fragment of (a) under stringent hybridization conditions of 6X SSC (1M NaCl), 40 to 45 % formamide, 1 % SDS at 37 °C, and a wash in 0.5X to 1X SSC at 55 to 60 °C; and

(c) an isolated nucleic acid fragment that is completely complementary to (a) or (b).

4. **(Currently Amended)** An isolated nucleic acid sequence encoding a nitrilase enzyme selected from the group consisting of SEQ ID NO:4, [SEQ ID NO:13], and SEQ ID NO:15[, and SEQ ID NO:16].

5. **(Currently Cancelled)**

6-8. **(Previously Withdrawn)**

9. **(Previously Amended)** A chimeric gene comprising the isolated nucleic acid fragment of any of Claims 1, 2, 3, or 4 operably linked to suitable regulatory sequences.

10. **(Currently Amended)** A plasmid pSW91 contained in *E. coli* SW91 having the designation ATCC PTA-1175, a plasmid pnit4 contained in *E. coli* DH5  $\alpha$ : pnit4 having the designation ATCC PTA-1176, a plasmid pnitex2 contained in *E. coli* SS1001 having the

designation ATCC PTA-1177, or a plasmid pnitex2 contained in either *E. coli* SS1002 or in *E. coli* SS1011.

11. **(Original)** An expression cassette comprising the chimeric gene of Claim 9.
12. **(Original)** The expression cassette of Claim 11 selected from the group consisting of the plasmids pSW91, pnit4, and pnitex2.
13. **(Original)** A transformed microorganism comprising the chimeric gene Claim 9.
14. **(Original)** A transformed microorganism comprising the plasmid of Claim 10.
15. **(Original)** A transformed microorganism comprising the expression cassette of Claim 11.
16. **(Original)** The transformed microorganism of Claim 15 wherein the expression cassette is chromosomally integrated.
17. **(Original)** The transformed microorganism of Claim 16 further comprising suitable regulatory sequences.
18. **(Original)** The transformed microorganism of Claim 17 wherein the suitable regulatory sequences comprise
  - a) at least one promoter selected from the group consisting of the tryptophan operon promoter P<sub>trp</sub> of *E. coli*, a lactose operon promoter Plac of *E. coli*, a P<sub>tac</sub> promoter of *E. coli*, a phage lambda right promoter P<sub>R</sub>, a phage lambda left promoter P<sub>L</sub>, a T7 promoter, a promoter of the AOX1 gene from *Pichia pastoris*, and a promoter of the GAP gene from *Pichia pastoris*, or is at least one strong promoter selected from the group consisting of *Comamonas*, *Corynebacterium*, *Brevibacterium*, *Rhodococcus*, *Azotobacter*, *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Salmonella*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Pichia*, *Zygosaccharomyces*, *Kluyveromyces*, *Candida*, *Hansenula*, *Dunaliella*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacteria*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Rhizobium*, and *Streptomyces*, and
  - b) at least one ribosome binding site from a phage lambda CII gene or selected from the group consisting of ribosome binding sites from a gene of *Comamonas*, *Corynebacterium*, *Brevibacterium*, *Rhodococcus*, *Azotobacter*, *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Salmonella*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Kluyveromyces*, *Candida*, *Hansenula*, *Dunaliella*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacteria*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Rhizobium*, and *Streptomyces*.
19. **(Original)** The transformed microorganism of Claim 18, wherein the host microorganism is selected from the group consisting of *Comamonas*, *Corynebacterium*, *Brevibacterium*, *Rhodococcus*, *Azotobacter*, *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Salmonella*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Zygosaccharomyces*,

*Pichia, Kluyveromyces, Candida, Hansenula, Dunaliella, Debaryomyces, Mucor, Torulopsis, Methylobacteria, Bacillus, Escherichia, Pseudomonas, Rhizobium, and Streptomyces.*

20. **(Currently Amended)** A transformed microorganism selected from the group consisting of:

- (a) *E. coli* SW91 having the designation ATCC PTA-1175;
- (b) *E. coli* DH5 $\alpha$ : pnit4 having the designation ATCC PTA-1176;
- (c) *E. coli* SS1001 having the designation ATCC PTA-1177; [and]
- (d) *E. coli* SS1002 containing plasmid pnitex2; and
- (e) *E. coli* SS1011 containing plasmid pnitex2.

Claims 24. through 45. **(Withdrawn)**

46. **(Original)** The transformed microorganism of Claim 19, wherein the host microorganism is *E. coli* strains MG1655 (ATCC 47076), W3110 (ATCC 27325), MC4100 (ATCC 35695), or W1485 (ATCC 12435) .

47. **(Currently Amended; Previously Added)** An isolated nucleic acid fragment encoding a polypeptide having the amino acid sequence of SEQ ID NO:5 [~~or SEQ ID NO:14~~].

48. **(Currently canceled).**

49. **(New)** The nucleic acid fragment of Claim 2 wherein the first nucleotide sequence encoding a polypeptide with nitrilase activity has at least 90 % identity as determined by the Needleman and Wunsch algorithm under default parameters compared to a polypeptide encoded by the sequence identified in SEQ ID NO:5.